

Center for Regulatory Services, Inc.

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April 15, 2015

CBIC Control Number

364764

U.S. Environmental Protection Agency – East

Attn: TSCA Section 8(e)

Room 6428

1201 Constitution Avenue, NW

Washington, DC 20004

SUBJECT: TSCA 8(e) Notification
Chemical Name: Fluorinated Surfactant
Trade Name: Dainaflow
LVE L-95-186

2015 APR 22 AM 10:53

RECEIVED
OPPT CBIC

The enclosed ecotox study that came the attention of JSR Micro, Inc., April 13, 2015, for the subject substance that is identified in Low Volume Exemption L-95-186.

The results of the Ecotox Testing of the subject substance were inconclusive, i.e., no mortality observed at the concentration limit (50 mg/L) for Daphnia and for Algae EC50 mg/L.

Please feel free to contact the undersigned if you have any questions or if we can provide additional information.

Sincerely,



William A. Olson, Ph.D.

Agent

JSR Micro, Inc.

WAO:gbt

JSR-8E-DAINAFLOW

Enclosures –

Report Ecotoxicity Testing Dainaflow(9 pages)

cc: T. Ozag, JSR (w/o Enclosures)

Report Ecotoxicity testing Dainaflow JSR Micro

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1. Dainaflow

Solubility of Dainaflow

The solubility of Dainaflow in water is extremely low. At a concentration of only 0,5mg/l the different algae replica's demonstrated a high variety in cell density, illustrative for the (in)solubility issue (see below)

Dainaflow in solvent Miltate

We opted to solubilize Dainaflow in a solvent. Dainaflow is used at JSRMicro with the solvents miltate and neotate. Because of the lower toxicity of miltate- as seen from the provided ecotox data- we opted for miltate as a solvent. Because of this approach, an extra solvent test was performed (with only miltate).

Assessment of solubility of Dainaflow in miltate

We first assessed the solubility range, taking into account the fact we have to dilute back the solution in aqueous solution.

The solubility of Dainaflow in miltate is very high. However, if diluted back into water, precipitates were observed already at low dilution. The maximal solubility of Dainaflow in the solution was about 100mg/liter as observed by visual inspection. We therefore did exposure between 0 and 100mg/ml

Miltate seems not to be bio-compatible (as shown in the results section), illustrated by an impact on the different test organisms.

Dainaflow in bio-compatible solvents

Solubility of Dainaflow was also assessed in different solvents of which it was known not to have any impact on the test-organism at a concentration of max 0.01% (V/V). Different solvents were tested- acetone, ethanol, DMSO

The highest solubility was obtained in acetone at a concentration of 1000mg Dainaflow/ml acetone. The highest dilution was determined to be 5 µl/100ml. As such the maximal Dainaflow concentration is 50mg/ml. The concentration gradient was between 0 and 50mg/ml.

1.2. Dainaflow and whole organism testing

1.2.1. Dainaflow and Daphnia

Daphnia tests were performed according to OECD 202- protocol

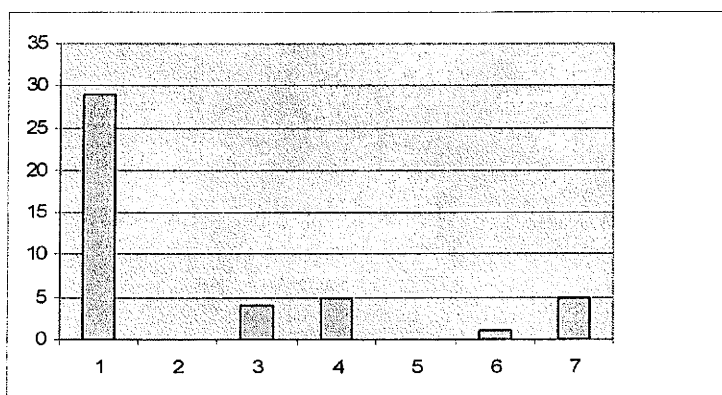
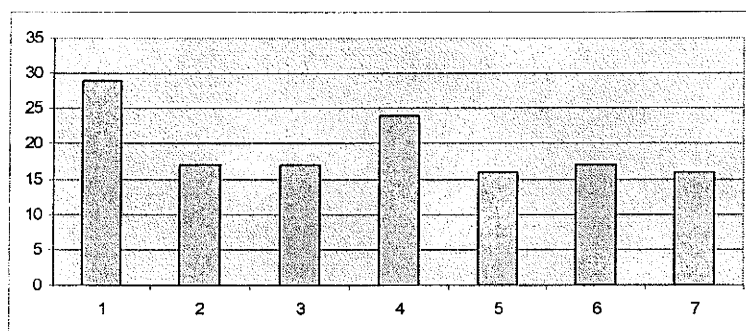
Acute testing of Daphnia with Dainaflow in miltate

Miltate does have a severe impact on Daphnia growth and survival.

The used solvent concentration of 0.1% (1ml/L) had a severe impact on survival after 24 h and 48h (as shown in Graph 1). This hampers the exact assessment of the toxicity in this solvent.

Graph1: number of Daphnia after 24h and 48h with miltate as solvent

(Y-axis: Number of living daphnids)



- | | | |
|---------------------|---------------|------------|
| 1. Negative Control | 4. 25 mg Df/L | 7. 100mg/L |
| 2. Solvent control | 5. 50 mg Df/L | |
| 3. 5 mg Dainaflow/L | 6. 75 mg Df/L | |

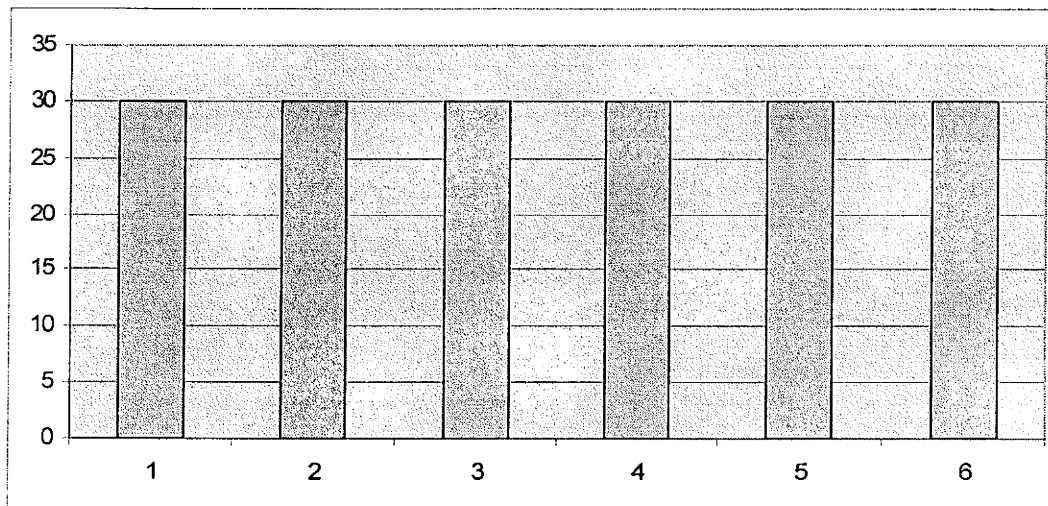
Because of the impact of the solvent, biocompatible solvents were also used that has a lesser impact on Daphnia growth and still allows 'high' solubility of Dainaflow. As explained before, acetone allowed the highest solubility.

Acute testing of Daphnia with Dainaflow in acetone

Tests were performed with Dainaflow in acetone. The highest solubility was 50 mg/ml.

Graph2: number of Daphnia after 24h and 48h with acetone as solvent

(Y-axis: Number of living daphnids)



- | | |
|---------------------|-----------------|
| 1. Negative Control | 4. 12.5 mg Df/L |
| 2. Solvent control | 5. 25 mg Df/L |
| 3. 5 mg Df/L | 6. 50mg Df/L |

Conclusion

No mortality was observed for any of the concentrations tested. Therefore it can be concluded that the compound is not toxic or that the LC50 is beyond the solubility range.

1.2.2. Algae test

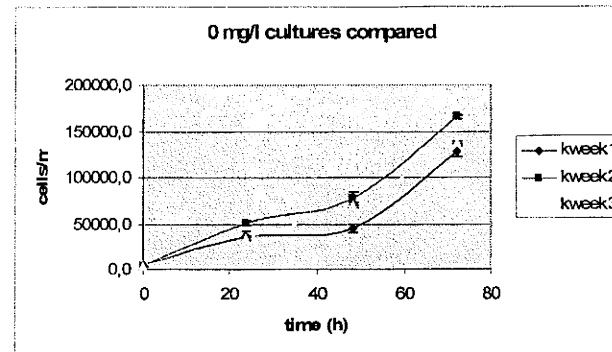
Algae tests were performed according to OECD 201. The used algae species are *Chlamydomonas reinhardtii* or *Pseudokirchneriella subcapitata* which are both accepted by OECD and have a similar sensitivity for most classes of compounds

Test of algae with Dainaflow in water

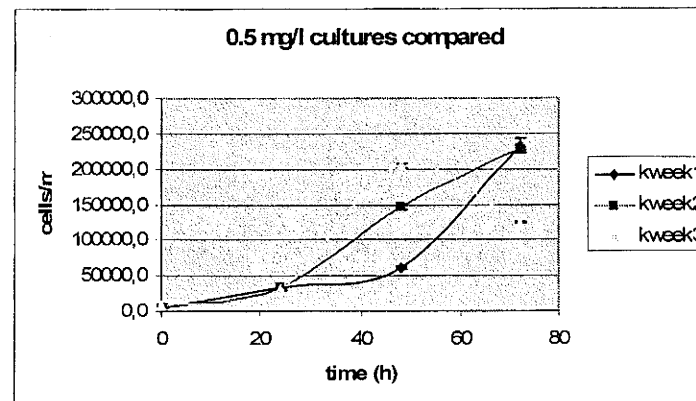
Dainaflow was tested in an algae toxicity test. The algae species for this testing was *Chlamydomonas reinhardtii*. Cell density was measured by Coulter counter.

The solubility in water of Dainaflow is extremely low. At a concentration of only 0,5mg/l the different algae replica's demonstrated a high variety in cell density, illustrative for the (in)solubility issue. The graphs show the cell densities of the replica's for the control and the exposed cells. The replica's of the control cells are comparable, the replica's of the exposed cells did show a high variety

Graph 3 : Cell densities of three replica's of control cells (0mg/liter Dainaflow)

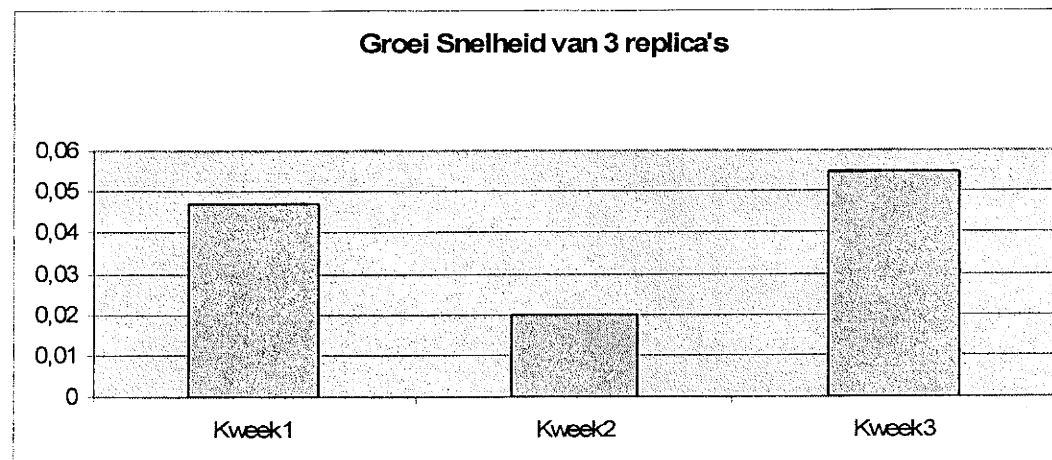


Graph 4: Cell densities of three replica's exposed cells with 0,5 mg Dainaflow/liter



This was also in accordance with the growth rate of the different replica's as shown in the graph5

Graph 5: Average growth rate of the three replica's



Test of algae with Dainaflow in miltate

For the reasons mentioned above, we opted to solubilize Dainaflow in a solvent. Dainaflow is used at JSRMicro with the solvents miltate and neotate. Because of the lower toxicity of miltate- as seen from the provided ecotox data- we opted for miltate as a solvent. For this approach, we also have to do an extra solvent control (with only miltate).

Determination of solubility of Dainaflow in miltate

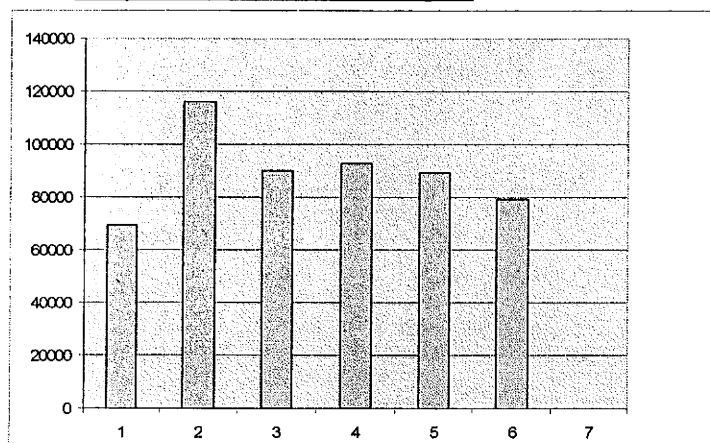
We first did assess the solubility range, taking into account the fact we had to dilute back in aqueous solution.

The solubility of Dainaflow in miltate is very high. However, if diluted back in water, precipitates were observed already at low dilution. The maximal solubility of Dainaflow in the solution was about 100mg/liter as observed by visual inspection. We therefore did exposure between 0 and 100mg/ml

Algae test with Miltate/Dainaflow

Miltate appears to be a very good nutrient source for algae. Cell density was much higher for the solvent control. And increase amount of Dainaflow had only a marginal effect on cell density

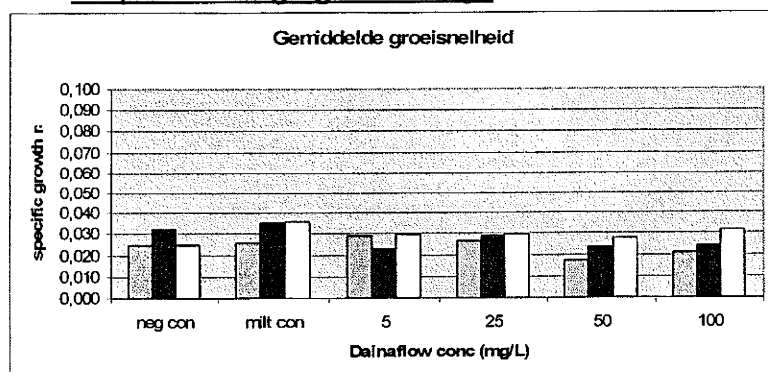
Graph 6: Cell density of Algae



- | | |
|------------------------|---------------|
| 1. Negative controle | 4. 25mg Df/L |
| 2. Solvent control | 5. 50 mg Df/L |
| 3. 5mg Dainaflow(Df)/L | 6. 100mg Df/L |

This was also in accordance with the growth rate of the different replica's as shown in the graph 5

Graph 7: Average growth range



On the basis of this experiment, because of the low solubility of Dainaflow, one can just make a rough estimation of Dainaflow toxicity (like EC50 >100mg/L)

Therefore it could be very useful to test other solvents (like DMSO, Ethanol, Acetone) to see whether the solubility range could be further increased. (Other algae species could also be useful to assess the nitrification aspect)

Test of algae with Dainaflow in biocompatible solvent Acetone

Algae toxicity test

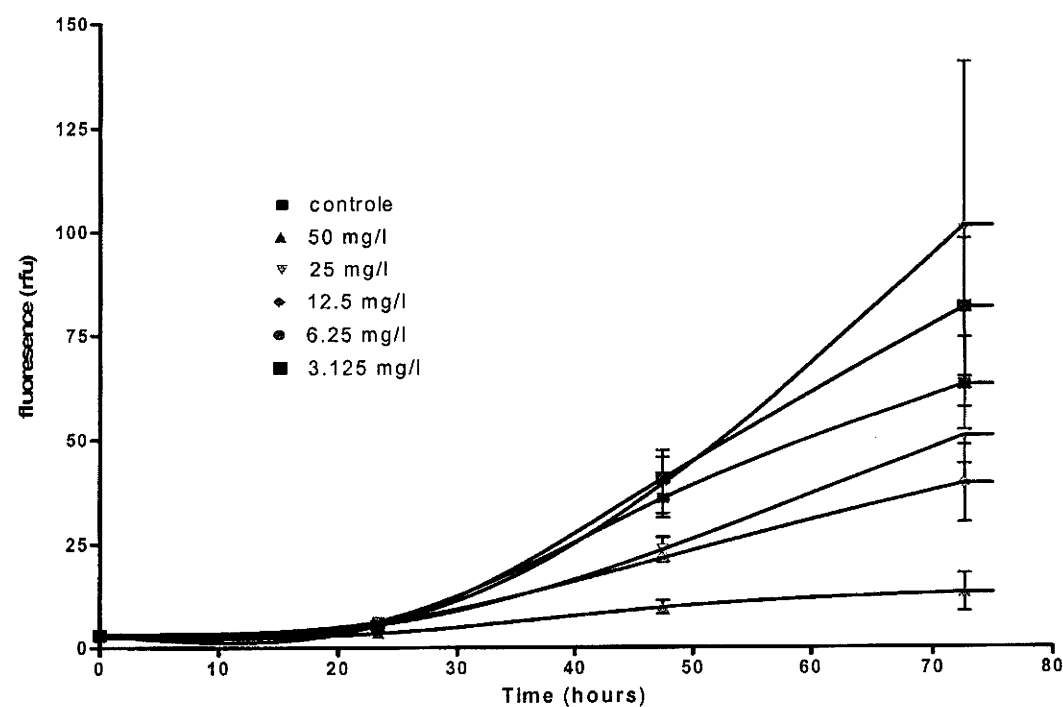
Test was performed according the OECD guideline 201 for testing of chemicals, Alga, Growth Inhibition test. The species used is *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*). The test was carried out in a miniaturised system using glass vessels with a 1.5 ml volume. Cell concentration is determined every 24 hours using a fluorimeter.

Results

Growth curves

Graph 8: Growth curves of algae with increasing Dainaflow concentration

GROWTH CURVES DIANAFLOW



Growth inhibition through comparison of growth rates

conc(mg/l)	μ	stdev μ	% inhib	stdev	N
3.13	0.0485637	0.0045968	-13.1198	17.78263	3
6.25	0.0352024	0.0061102	18.00291	20.1779	3
12.50	0.0479073	0.0099378	-11.5908	27.14154	3
25.00	0.0399666	0.0041797	6.905441	17.14326	3
50.00	0.0235137	0.0064901	45.22943	21.6156	3
c	0.0429312	0.0060434			5

Conditions for the validity of the test

pH is fixed to 8-8.1 before testing. pH is measured again after test. An explanation should be provided if pH deviations of more than one PH unit are observed.

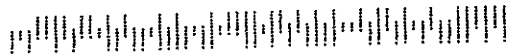
pH criterion is met.

Growth criterion: (16 fold growth is required) is sufficiently met.

well	RFU72/RFU0
C1	27
C2	23
C3	17

Conclusion

Only the highest concentration (50 mg/l) causes significant growth inhibition. Calculation of a sigmoidal dose response curve is not possible since data covering the upper range of this curve are absent because of the solubility issue. A standard EC50 calculation with nonlinear regression is therefore not possible. 50 mg/l Dainaflow causes however a 45% growth inhibition, therefore it can be stated that the EC50 concentration approximates 50 mg/l. The no observed effect concentration for this test is 25 mg/L. Further testing in the range 5 to 50 mg/l might give more detailed picture on the exact concentration where acute effect starts to occur.



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